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Docket No. 95-97

PATENT APPLICATION TRANSMITTAL LETTER

To the Commissioner of Patents and Trademarks:

Transmitted herewith for filing under 35 U.S.C. 111 and 37 C.F.R. §1.53 is the patent application of:
Guang-Jer Wuentitled: DIAGNOSTIC FOR METASTATIC PROSTATE CANCER.

Enclosed are:

26 pages of written description, claims and abstract
 6 sheets of drawings
 17 pages of Sequence Listing as paper copy
 an assignment of the invention to _____
 an executed declaration of the inventors and power of attorney
 a certified copy of a _____ application
 associate power of attorney
 a verified statement to establish small entity status under 37 CFR §1.9 and §1.27 (executed)
 information disclosure statement
 preliminary amendment
 Sequence Listing diskette, Statement under 37 C.F.R. 1.821-1.824

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Washington, D.C. 20231

September 1, 2000
Date *Nina Reel*
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CLAIMS AS FILED				
	Number Filed	Number Extra	Rate	Fee
BASIC FEE			\$690	\$690
TOTAL CLAIMS	19 -20 =		x \$18	0
INDEPENDENT CLAIMS	4 - 3 =		x \$78	\$78
MULTIPLE DEPENDENT CLAIM PRESENT			x \$260	0
* Number extra must be zero or larger				TOTAL \$768

If applicant has small entity status under 37 C.F.R. 1.9 and 1.27, then divide total fee by 2, and enter amount here.	SMALL ENTITY TOTAL	\$384
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Other _____

Benefit of Prior U.S. Application(s) (35 USC 120)

Applicant claims priority under 35 USC 120 to the following application(s):

PCT US99/04850 filed 2 March 1999 and designating the United States

Benefit of Prior U.S. Provisional Application(s) (35 USC 119(e))

Applicant claims priority under 35 USC 119(e) to the following application(s):

60/076,664 filed 3 March 1998

Benefit of Prior Foreign Application(s) (35 USC 119)

Applicant claims priority under 35 USC 119 to the following application(s):



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Docket No.: 95-97
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Applicant or Patentee: Guang-Jer Wu
Serial or Patent No.:
For: Diagnostic for Metastatic Prostate Cancer

Attorney's # : 95-97 US
Filed or Issued:

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: EMORY UNIVERSITY
ADDRESS OF ORGANIZATION: 2009 Ridgewood Drive
Atlanta, GA 30322

TYPE OF ORGANIZATION

- UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION LOCATED IN ANY COUNTRY
- AN ORGANIZATION OF THE TYPE DESCRIBED IN SECTION 501(c)(3) OF THE INTERNAL REVENUE CODE OF 1954 (26 U.S.C. 501(c)(3)) AND EXEMPT FROM TAXATION UNDER SECTION 501(a) OF THE INTERNAL REVENUE CODE (26 U.S.C. 501(a))
- ANY NONPROFIT SCIENTIFIC OR EDUCATIONAL ORGANIZATION QUALIFIED UNDER A NONPROFIT ORGANIZATION STATUTE OF A STATE OF THIS COUNTRY (35 U.S.C. 201(i))
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I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees under §41(a) and (b) of Title 35, United States Code, with regard to the invention entitled "Diagnostic for Metastatic Prostate Cancer"

by inventor(s) Guang-Jer Wu

described in the specification filed herewith

application serial no. _____ filed _____
 patent no. _____ issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(e). NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Mary L. Severson
TITLE IN ORGANIZATION: Director, Office of Technology Transfer
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SIGNATURE Mary L. Severson

DATE Aug 24, 2000

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OD

APPLICATION FOR LETTERS PATENT

Inventor: Guang-Jer Wu

DIAGNOSTIC FOR METASTATIC PROSTATE CANCER

CERTIFICATE OF MAILING

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September 1, 2000

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DIAGNOSTIC FOR METASTATIC PROSTATE CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of International Application PCT US99/04850, filed
5 March 2, 1999 and designating the United States, which application claims priority from
United States Provisional Application No. 60/076,664 filed March 3, 1998.

ACKNOWLEDGMENT OF FEDERAL RESEARCH SUPPORT

Not applicable.

BACKGROUND OF THE INVENTION

10 Prostate cancer accounts for about 1 in 10 cancer cases in the United States, and it is the most often diagnosed cancer in males [Henderson et al. (1991) *Science* 254, 1131-1138]. While in many affected patients, the tumors are slow-growing and nonmetastatic, in others the malignant prostate tumors are aggressive and metastasize. When prostate cancer metastasizes, the prognosis for the patient is poor, especially without treatment.

15 To date, the most frequently used test for prostate cancer is the serum level of prostate specific antigen (PSA) and the radionuclide bone scan for detecting prostate cancer metastases before definitive therapy is initiated. However, the elevated level of PSA in serum is not predictive of the pathologic stage of the prostate cancer or the presence of metastatic disease. PSA, a serine protease, is not exclusively expressed in the epithelial cells of
20 metastatic prostate cancer, but it is also expressed in normal epithelial cells, primary tumors and benign prostate hyperplasia.

5 The altered expression of cell-adhesion molecules has been correlated with metastasis of many cancers. Low or no expression of E-cadherin, a cell-adhesion molecule, has been found in high-grade prostate carcinoma, and this indicates a poor prognosis for those prostate cancer patients. However, the absence of an antigen is not very useful as a diagnostic marker for cancer metastasis.

MUC18 is a glycoprotein of about 113 kDa which serves as a cell adhesion molecule on the surface of melanoma cells, and it has been correlated with the ability of melanomas to metastasize [See, e.g., Lehmann et al. (1989) *Proc. Nat. Acad. Sci. USA* 86, 9891-9895; Luca et al. (1993) *Melanoma Res.* 3, 35-41; Johnson et al. (1996) *Curr. Top. Microbiol. Immunol.* 10 213, 95-105; Xie et al. (1997) *Cancer Res.* 57, 2295-2303; Tang and Honn (1994-1995) *Invasion Metas.* 14, 109-122; Rummel et al. (1996) *Cancer Res.* 56, 2218-2223]. MUC18 is also known as MCAM and CD146. MUC18 carries a carbohydrate modification known as HNK-1 or CD57 [Shih et al. (1994) *Cancer Res.* 54, 2514-2520]. Besides being associated with melanoma cells' ability to metastasize, MUC18 is also associated with normal vascular tissue, and on the smooth muscle of venules, and it expresses sporadically on capillary epithelium [Johnson, J. (1994-1995) *Invasion Metas.* 14, 123-130].

15 There is a longfelt need in the art for an improved diagnostic test for metastatic prostate cancer so that appropriate therapy can be initiated as soon as possible and so that the number of false positive results can be minimized.

20 **SUMMARY OF THE INVENTION**

The present invention provides an improved diagnostic test for prostate cancer which has a relatively high potential for metastasis or which has metastasized. This allows the physician to choose appropriate surgical, chemotherapeutic or radiation treatment regimens. This improved assay is based on the correlation of high levels of expression of the MUC18 25 coding sequence as measured by MUC18 mRNA or MUC18 protein. This expression can be detected at the transcriptional level, where mRNA levels are monitored, or detection of the MUC18 gene product at the translation level can be determined, for example, through the use of an immunoassay for the MUC18 protein. The source of the material for these tests is prostate biopsy tumor tissue (e.g., from a needle biopsy) from a patient needing a

determination of the metastatic potential of a prostate tumor or from cells from a prostate tumor.

5 Relative levels of transcriptional expression (mRNA) of the MUC18 coding sequence can be determined by Northern hybridization analysis or by quantitative reverse transcription polymerase chain reaction (RT-PCR) in normal and neoplastic prostate tissue samples and in biopsy material.

10 Translational expression of MUC18 can be determined by any of a number of adaptations of an immunoassay using antibody specific for the MUC18 cell surface antigen. The relative level of MUC18 can be determined by standard immunoassays using a MUC18-specific antibody preparation and a detection system suitable for the assay. Immunoassays can include, but are not limited to, immunofluorescence assays, radioimmunoassays, enzyme-linked immunosorbent assays, and Western (immuno) blot assays. In the context of the present invention, relative amounts of the MUC18 protein are determined in tissue samples (e.g., biopsy material).

15 It is a further object of the present invention to provide an antibody which inhibits prostate cancer metastasis. In particular, antibody specific to MUC18 prevents metastasis of prostate cancer cells.

20 Additional objects include vectors directing the expression of an immunogenic fragment of human MUC18 and the corresponding recombinantly expressed protein. As specifically exemplified, an immunogenic fragment of human MUC18 is encoded by the Pvull to XhoI fragment within the sequence given in Tables 1A-1B.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results for RT-PCR amplified human MUC18 cDNA (left panel) and cloning of the whole cDNA and its fragments into a GST-fusion protein expression vector (right panel). RT-PCR amplification of human MUC18 cDNA from the poly(A)+RNA isolated and purified from a human melanoma cell line, Sk-Mel-28. Left panel: Lane (a) shows the expected PCR product of 1957 bp (as indicated by an arrow head). Lane (m1) shows the 1 kb ladders and lane (m2) the 123-bp ladders as DNA molecular size markers. Right panel: The plasmid map of the cloned whole human MUC18 cDNA and three fragments in a GST-fusion expression system.

Fig. 2 illustrates Northern blot analysis of expression of human MUC18 in different prostate cancer cell lines. Poly(A)+RNA was isolated from human melanoma cells SK-MEL-28 (SK), human melanocyte (M), and human prostate cancer cells PC-3 (PC-3), DU145 (DU), TSU-PR-1 (TSUPR1), and LNCAP (LNCAP). The size of the human MUC18 mRNA is 3.3 kb. The amount of poly(A)+RNA (2.5 to 10 μ g) is indicated as a number on top of each lane.

Fig. 3 depicts recombinant human MUC18-middle fragment. The plasmid map is shown in Fig. 1. The left panel shows the PAGE result and right panel the Superdex column purification. The GST-human MUC18 middle fragment fusion protein is shown after IPTG induction (a & b, indicated by a triangle on the left of the left panel). The fusion protein was first purified through a glutathione-Sepharose affinity column and then cleaved with the HRV-3C protease (left panel, lanes c-e). The affinity-purified recombinant human MUC18-middle fragment was then further purified through a Superdex column (right panel) to remove high molecular weight contaminants (peaks I and II, fractions 3-13). The final recombinant human MUC18-middle fragment protein is about 22 kDa (peak III, fractions 14-20), as indicated by a triangle on the right in the left panel.

Fig. 4 shows pGEX-6P, commercially available from Pharmacia Biotech, Piscataway, NJ. The specific multiple cloning site (MCS) sequences for pGEX-6P-1 (SEQ ID NO:11, encoded amino acids, SEQ ID NO:12), pGEX-6P-2 (SEQ ID NO:13, encoded amino acids, SEQ ID NO:14) and pGEX-6P-3 (SEQ ID NO:15, encoded amino acids, SEQ ID NO:16) are provided.

Fig. 5 provides diagrammatic illustrations of the whole cDNA sequence of human MUC18, its N-terminal fragment, middle fragment and C-terminal fragment, as cloned into the pGEX-6P-1 vector are provided. These fragments as cloned result in the expression of the N-terminal, middle and C-terminal fragments of the MUC18 protein. See also Tables 1A-25 1B for the locations of the relevant restriction sites in the cDNA sequence for human MUC18 and SEQ ID NO:1 for sequence.

Fig. 6 illustrates the results of Western blot analysis of huMUC18 protein expression in four prostatic cancer cell lines. Cellular extracts of four prostatic cancer cell lines were prepared, and the proteins were size-separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The anti-huMUC18 antibodies generated by immunization of 30 the recombinant huMUC18-middle portion protein in chicken were used for Western blot

analysis. SK stands for cellular lysate prepared from human SK-Mel-28 cells (a), Tsu-PR1 cells (b and c), DU145 for DU145 cells (d and e), PC-3 for PC-3 cells (f and g), and LNCaP for LNCaP.FGC cells (h and i). The number over each lane indicates the amount of protein (μ g) loaded in each well. The huMUC18 protein band is indicated with an open triangle.

5 The numbers on the right-most lane indicate the protein molecular weight (kDa) rainbow markers (RPN800, Amersham).

10 Fig. 7 shows the results of Western blot analysis of human MUC18 protein in normal prostatic gland, normal primary human prostatic epithelial cells, and tissues of a patient with malignant prostatic cancer. Cellular lysates were prepared from normal prostatic gland (b), cultured normal primary prostatic epithelial cells (c), and prostatic cancerous tissues from a patient with malignant prostatic cancers (e-g). Cellular extracts prepared from human SK-Mel-28 cells (a) and from DU145 cells (d) were shown as the positive controls. 20 μ g protein of each lysate was loaded per well. The numbers on the right-most lane indicate the protein molecular weight (kDa) rainbow markers (RPN800, Amersham).

15 DETAILED DESCRIPTION OF THE INVENTION

20 Prostate cancer accounts for about 10% of all cancer cases in the United States. It is now the most frequently diagnosed cancer in American males [Rinker-Schaeffer et al. (1993) *Cancer and Metas. Rev.* 12, 3-10]. In some patients, prostate cancers metastasize rapidly, killing the patient within one year of the initial clinical presentation. In contrast, some other prostate cancer patients show a relatively slow growth of the malignant tumor without metastasis. The majority of the histologically localized prostate cancers remain subclinical and never require treatment. Prostate cancer, when truly localized, can be cured by radical prostatectomy. While in many affected patients, the tumors are slow-growing and nonmetastatic, in others prostate cancer takes a more aggressive course. Unfortunately, 25 metastatic prostate cancer is a fatal disease without treatment.

30 At present, the test for serum levels of prostate specific antigen (PSA) and the radionuclide bone scan are the only diagnostic tests available before therapy is initiated. However, an elevated level of PSA observed in serum is not predictive of the pathological state of prostate cancer, nor is it correlated with metastatic prostate cancer. This is, at least in part, because PSA, a serine protease, is not exclusively expressed in the epithelial cells of

metastatic prostate cancer, but it is also expressed in normal epithelial cells, primary prostate cancerous tumors and benign prostatic hyperplasia [Wood et al. (1994) *Cancer* 74, 2533-2540]. To date, it has not been possible to predict whether histologically detected localized tumors are likely to progress to clinical cancer or when these localized tumors are likely to metastasize to other sites within the body. Thus, there is an urgent need for biochemical markers which serve to identify prostate cancers which have progressed to a stage which requires immediate surgical removal and/or additional chemotherapeutic or radiation therapeutic treatments, i.e., those cancers which are likely to metastasize.

To identify a diagnostic marker which is improved over PSA, it is crucial to understand the biochemical differences between the malignant state and the benign state and between tumor cells with high and low metastatic potential. Overexpression and underexpression of certain cell adhesion molecules at the cell surface has been proposed to reflect metastasis of several cancers [Tang and Honn (1994-1995) *Invasion Metas.* 14, 109-122]. For example, the low expression of E-cadherin has been correlated with poor prognosis of prostate cancer [Rinker-Schaeffer et al. (1993) *supra*]. The present invention provides a positive correlation between the level of MUC18 expression and high metastatic potential of prostate cancer cells.

The human MUC18 (huMUC18) cDNA sequence (see Table 1A) obtained by the present inventors is different from the huMUC18 cDNA sequence given in GenBank Accession No. N28882 [Johnson et al. (1994)]. The deduced amino acid sequence of huMUC18 cDNA given in Table 1A was identical to the huMUC18 sequence deposited in GenBank by Johnson's group except seven amino acid residues. This discrepancy of amino acid sequence may be due to allelic differences. However, the amino acid sequence of the inventor's huMUC18 (646 amino acids) was very different from that published by Johnson's group in 1989 (603 amino acids), which 1989 sequence appears to contain sequence errors in the huMUC18 cDNA.

The human MUC18 cDNA sequence disclosed in Table 1A was amplified by RT-PCR from poly(A)+RNA, which was isolated from the human melanoma cell line SK-Mel-28. The cDNA was cloned into the pGEM-T-easy vector (Promega, Madison, WI). The DNA sequence of the huMUC18 cDNA was determined by rapid DNA sequencing using ABI prism dye terminator cycle sequencing ready reaction kit (Perkin-Elmer) with various

huMUC18 specific primers in an automated sequencer of ABI 373XL system. The Lasergen and GCG programs were used for comparison of nucleotide and amino acid sequences of the huMUC18 cDNA.

5 A cDNA sequence of a human MUC18 clone has been published in GenBank under Accession No. M 28882 (See Table 1B). The translation initiation codon (ATG) is underlined, as is the translation termination codon (TAG). The PvuII (CAGCTG) and XhoI (CTCGAG) restriction sites are boxed, and cut sites are indicated by vertical arrows.

10 Table 1C (see also SEQ ID NO:5) shows the human MUC18 sequence as modified to introduce a BamHI site (GGATCC) just upstream of the translation start site to facilitate cloning. The translation initiation (ATG) and termination (TAG) codons are boxed. The long arrows near the 5' and

TABLE I A

HUMAN MUC18 cDNA SEQUENCE WITH DEDUCED AMINO ACID SEQUENCE

TABLE I B

HUMAN MUC18 cDNA (GENBANK ACCESSION NO. M28882).

TABLE I C

HUMAN MUC18 cDNA WITH 5' MODIFICATION TO FACILITATE CLONING

BamHT

ERGA

3' ends indicate primer positions (BF1 and ER6a, respectively). The cut site for BamHI within its recognition sequence (GGATCC) is indicated with a vertical arrow.

The GenBank M 28882 sequence (given in SEQ ID NO:3) is identical to a human MUC18 cDNA clone (huMUC18) from human SK-Mel-28 cells, a human malignant melanoma cell line which produces relatively high levels of the MUC18 protein. This sequence is slightly different from the huMUC18 previously published [Johnson and Rummel (1996) in *Immunology of Human Melanoma*, ed, Maio, M., IOA Press, Washington, DC, pp. 31-38; Lehmann et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 9891-9895; Luca et al. (1993) *Melanoma Res.* 3, 35-41]. The two cDNAs have three stretches of amino acid (aa) residues that are different, such as 19 aa and 17 aa at the N-terminal portion and 17 aa near the C-terminal portion. Furthermore, the published human MUC18 cDNA sequence was missing 42 amino acids at the C-terminal end (see SEQ ID NO:4). The human and murine cDNAs have 74.5% identity in the deduced amino acid sequences. The 3'-end primer used previously, ER6, did not include the last few codons and the termination codon. To re-clone the intact correct human MUC18 cDNA, a correct new 3'-primer, ER6a, for amplifying the intact human MUC18 cDNA was designed (see hereinbelow).

Efforts to express the recombinant huMUC18 protein in the pCal-n expression system (Stratagene, La Jolla, CA) in *E. coli* failed. Finally, the expression was possible by using a GST-fusion protein expression system. The huMUC18 cDNA was cloned in the PGEX-6p-1 vector (Pharmacia), a small amount of the nearly intact MUC18 protein in *E. coli* was expressed. Fortunately, the sequence of the middle portion of the huMUC18 cDNA was correct, and it was then used for making recombinant protein in *E. coli*. Only when the middle 166 amino acid portion encoded by the cDNA, but not the N-terminal or C-terminal portions, was used for expression, over-expression of the recombinant protein was possible. One pair of primers: BamHI-HMUC18-pvuII (28-mer, GGATCCCAGCTGGTTAAAGAAGACAAAG) (SEQ ID NO:6) and HMUC18-xhoI (27-mer, CTGGAACTCGAGGTCTGGCTACTCTC) (SEQ ID NO:7) were used for PCR-amplification of the region from PvuII to XhoI of the HuMUC18 cDNA. The amplified fragment was cloned into pGEM-T Easy vector. The DNA fragment that included the coding region from the PvuII site to the XhoI site was excised from the pGEM-T Easy recombinant plasmid by two restriction enzymes, BamHI and SalI, and cloned into the BamHI and SalI

cleaved pGEX-6P-1 vector. The recombinant HuMUC18-middle fragment after cleavage with PrScission protease and purification contained the following sequences (see also Table 1A and SEQ ID NO:2).

211

376

5 (Gly-Pro-Leu-Gly-Ser-)(SEQ ID NO:8)**Gln-Leu- Leu-Glu-Phe-Gln-(Asn-His).**
PGEX-6P-1 vector (Amino acids 211-376 of SEQ ID NO:2) pGEM T Easy vector

A 22 kDa protein fragment was expressed from the PvuII to XhoI fragment of the cDNA (see Tables 1A and 1C). A large-scale preparation of the recombinant human MUC18 "middle portion" fragment was carried out. More than 6 mg of the purified recombinant protein was obtained after purification through a glutathione-affinity column, cleavage with the HRV-3C protease, being eluted and concentrated, and further purification through a Superdex column in a Pharmacia FPLC system. After final concentration of the eluant, 6 mg of the "middle portion" recombinant protein was sent to Lampire Biological Laboratories to make polyclonal antibodies in chickens. High antibody titers were reported. Eggs are collected and IgY (chicken antibody protein) is purified from these eggs. After the titers of these purified IgY preparations are determined, they are used for immunological testing.

Using the MUC18-specific antibody preparation from chickens after immunization with the purified human recombinant huMUC18-middle fragment protein, the present inventor has shown that these antibodies can react with the human MUC18 protein expressed in human prostate cancerous cell lines and prostatic cancerous tissues by Western blot analysis. The results showed that the human MUC18 protein was only expressed in three metastatic prostate cancer cell lines (Tsu-PR-1, DU145 and PC-3), but not in one non-metastatic cell line (LNCaP.FGC). These results are consistent with the Northern blot analysis of the expression of huMUC18 mRNA in these prostatic cancer cell lines, described herein. The human MUC18 protein was weakly expressed in normal prostatic epithelial cells and in normal prostate gland, but highly expressed in human cancerous prostatic tissues, as shown by Western blot analysis of the extracts prepared from these tissues. See Fig. 6 for the results of Western blot analysis.

Further immunohistochemical analysis revealed that huMUC18 is expressed in the membrane of the expected special cell types, such as metastatic melanoma tissues, endothelial

cells, and smooth muscle cells. This indicates that the antibodies are very specific for huMUC18 antigen and work well with the formaldehyde-fixed, paraffin-embedded tissue sections. As expected, the antibodies did not react with any antigens of the normal secretory epithelial cells in the acini of the prostate gland. Interestingly, they react with the secretory epithelial cells in the acini of the prostate cancer tissues. The expression of human MUC18 protein (or antigen) only in cancerous epithelial cells, but not in normal epithelial cells, supports the use of human MUC18 as a diagnostic marker for the metastatic potential of prostate cancers.

Table 2 summarizes the results for MUC18 expression in four prostate cancer cell lines in comparison with pertinent results published by other groups. Expression of MUC18 in these cell lines is consistent with their low or no expression of E-cadherin and α -catenin and their extent of invasiveness *in vitro* and metastasis in nude mice. See Fig. 6 for Western blot analysis.

The present work has correlated relatively high levels of MUC18 with the ability of prostate cancer cells to metastasize. High levels of MUC18 expression were observed in the three metastatic prostate cancer cell lines TSU-PR1, DU145 and PC-3. MUC18 expression was not detectable in the LNCAP prostate cell line, which is not metastatic. Nonmetastatic prostate cancer cells and normal prostate cells produce no or barely detectable expression of MUC18 either as protein or mRNA. Experiments in which the LNCAP cell line is genetically engineered to express MUC18 at high levels demonstrate that when cells gain the capacity to express MUC18 at high levels, those cells gain the ability to metastasize.

Experiments in which the nonmetastatic prostate cancer cell line LNCaP.FGC is genetically engineered to express MUC18 at high levels demonstrate that when the cells gain the capacity to express MUC18 at high levels, the ability to metastasize is also gained. Thus, the relative level of MUC18 expression in prostate tumor tissue is correlated with the ability to metastasize, and measurement of MUC18 expression in prostate tumor biopsy tissue allows the medical practitioner to choose the most appropriate therapy for each prostate cancer patient, with high levels of MUC18 expression mandating an aggressive treatment strategy, likely including surgery, chemotherapy and/or radiation.

TABLE 2
RELATIVE LEVELS OF MUC18 EXPRESSION

	TSU-PR1	DU145	PC-3	LNCAP	Reference
5	E-cadherin expression	0	0.1	0.6	1.1 Morton et al. (1993) <i>Cancer Res.</i> 53,3585-3590
10	α -catenin expression	none	none	none	yes Morton et al. (1993)
	Total RNA (MUC18)	yes	yes	yes	none this work
	tumor growth in nude mice	yes (2000)	yes (500)	Yes (1400)	yes (2000) Passaniti et al. (1992) <i>International J. Cancer</i> 51,318-324
	Metastasis in nude mice	yes	yes	yes	none Lalani et al. (1992) <i>Cancer Metastasis Rev.</i> 16,29-66
	Invasiveness <i>in vitro</i>	yes (220)	yes (25)	yes (20)	none Passaniti et al. (1992)

Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with MUC18, may be made by methods well known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to, the methods described in U.S. Patent No. 4,816,567. Monoclonal antibodies with affinities of $10^8 M^{-1}$, preferably 10^9 to $10^{10} M^{-1}$ or more, are preferred.

20
25 Antibodies (polyclonal or monoclonal) specific for MUC18 are useful, for example, as probes for screening DNA expression libraries or for detecting the presence (and relative amounts) of MUC18 in a test sample, for example, prostate tumor biopsy tissue or a tissue slice of a metastatic prostate cancer, or cells in culture which were derived from a primary prostate cancerous tumor or a metastatic prostate cancer tumor. Desirably, the results are

normalized to cell number or to total cellular protein. Frequently, the polypeptides and antibodies are labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include, but are not limited to, radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, 5 magnetic particles and the like. United States Patents describing the use of such labels include, but are not limited to, Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and 10 the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218, Part I; Wu (ed.) (1979) *Meth Enzymol.* 68; 15 Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL 20 Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

25 All references cited in the present application are incorporated by reference herein to the extent that they are not inconsistent with the present disclosure.

The following examples are provided for illustrative purposes, and are not intended to limit the scope of the invention as claimed herein. Any variations in the exemplified articles and/or methods which occur to the skilled artisan are intended to fall within the scope of the 30 present invention.

EXAMPLES

Example 1. Recombinant MUC18 Production and Antibody Production

The human MUC18 cDNA (1970 bp, RT-PCR amplified fragment) and three sub-fragments have been cloned in-phase in a GST-fusion protein expression system, pGEX-6p-1 (Pharmacia), which contains the glutathione-S-transferase (GST) coding region as an affinity-tag for the inserted foreign protein at its C-terminus. Fig.1 and Fig. 5 show the four possible fusions: the whole region, the N-terminal fragment, the middle fragment, and the C-terminal fragment.

Only the middle fragment of the human MUC18 protein can be induced by IPTG to express in a high amount in *E. coli* K-12 strain BL-21. Thus, only this protein is further purified for immunization. When culture A_{600} reaches 0.6 (2 to 3 hours after 1/100 inoculation of an overnight culture in L-broth with ampicillin), the expression of the recombinant middle fragment of MUC18 protein fused to GST in recombinant *E. coli* is induced by addition of 0.1 mM of IPTG to 3-liter cultures (1.5 liters per 4-liter baffled flask). Two hours after addition of IPTG at 37°C, cells are harvested by centrifugation at 3,000 rpm (2,323 x g) for 20 min in a horizontal HG-4L rotor in Sorvall RC-3 centrifuge. The cell pellet is suspended in 40 ml of ice-cold PBS (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 2.7 mM KCl, and 140 mM NaCl, pH 7.3) and then lysed with a prechilled French pressure cell at 800 psi. The lysate is clarified by centrifugation for two to three times at 13,000 rpm (21,000 x g) for 30 min in SS-34 rotor in Sorvall RC-2 centrifuge. The protein concentration of the clear crude lysate adjusted to 10 mg/ml protein (about 60 ml) was used as the starting material for purification. The recombinant MUC18 proteins are purified from the clear crude lysate by batchwise adsorption to the Glutathione-Sepharose 4B affinity resin (about 20 ml of 50% slurry) by inversion on an inversion shaker at room temperature for 30 min. The GST portion of the fusion protein mediates the binding of the protein to the resin via the glutathione. After twice washing with 10 volumes (50 ml per 5 ml packed resin) of 1 X PBS and followed by twice washing with 1 X PreCision protease cleavage buffer (50 mM TrisHCl, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) to remove unbound proteins, the fusion protein on the resin is cleaved with 100 units of HRV-3C protease (PreCision protease, 2 units/μl, from Pharmacia) by rocking on an inversion shaker for 16 hours at 4°C. The resin was spun down at 2,000 rpm for 10 min in a Sorvall RC-32 centrifuge. The supernatant and three washings

(10 ml 1 X PBS per 10 ml resin), which contain the recombinant MUC18 protein, are then combined and concentrated by centrifuging through a Centricon-30 (Millipore/Amicon). The purity of the protein is characterized by SDS-PAGE (8 to 10% polyacrylamide gel, slab gel). The 70kDa contaminated protein is removed by passing through a Superdex 200 HR 10/30 column in 1 X PBS (void volume about 7 ml for a 20 ml packed column), and the fractions containing the recombinant middle fragment MUC18 protein (22 kDa) (eluted at about 15.5 ml) were pooled. Fig. 2 shows the SDS-PAGE results of recombinant huMUC18 protein in the GST-fusion system.

Six mg of protein is sent to Lampire Biological Lab. (Pipersville, PA) for immunizing three chickens. The anti-MUC18 antibody titers are determined by ELISA assay using the purified recombinant MUC18 as the antigen. Eggs collected during the period of high serum antibody titers are used for purification of chicken immunoglobulin IgY.

To confirm the association between MUC18 expression and metastatic ability of prostate cancer cells, the human MUC18 coding sequence is introduced into a non-metastatic, non-expressing human prostate cancer cell line (LNCAP), and clones with different levels of expression of MUC18 are isolated.

The human MUC18 cDNA has been cloned into pCR3.1 Uni (Promega, Madison, WI), a mammalian expression vector in which high levels of gene expression are driven by the human CMV-IE promoter. The human MUC18 cDNA is also cloned into a murine amphotrophic retrovirus expression vector, e.g. pZipNeoSVX [Cepko et al. (1984) *Cell* 37, 1053-1062] or LXSN [Miller and Rosman (1989) *BioTechniques* 7, 980-990], in which LTR drives gene expression.

These MUC18 recombinant vectors are used to transfect a human prostate cancer cell line which does not express MUC18, for example the LNCaP.FGC cell line [Umbas et al. (1992) *Cancer Res.* 52, 5104-5109; Iizumi et al (1987) *J. Urol.* 137: 1304-1306]. The vectors are introduced into the cultured cells by lipofection [Felgner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 7413-7417] or by electroporation [Potter, H. (1988) *Anal. Biochem.* 174:361-373]. G418-resistant clones are selected and purified [Yuo et al. (1992) *Intervirol.* 34, 94-104] in view of the kanamycin resistance coding sequence expressed under the control of the SV40 promoter in each of the vectors. Relative expression levels of MUC18 expression in

different clones are determined by western blotting with polyclonal antibodies (described herein). Metastatic abilities are determined as described herein.

Example 2. Determination of Metastatic Ability of Prostate Cancer Cells

The degree of motility and the invasiveness of prostate cancer cells are quantitated using published methods [Tucker et al. (1994) *Eur. J. Cell Biol.* 58, 28-290; Repesh, L.A. (1989) *Invasion and Metastasis* 9, 192-208]. The Costar transwell chamber contains an inner well with a porous polycarbonate membrane, with 3 μ m pore sizes in the bottom of the well. This is tightly fitted to the outer well.

To determine the motility of human prostate cancer cells, 0.5×10^5 cells are seeded in the top well. The cells remain on the top well because the poly carbonate membrane only allows medium to pass through freely. After seeding and attachment, the cells in the top well gradually migrate through the pores in the polycarbonate membrane to the bottom side of the membrane. Eventually some cells establish growth at the bottom side of the membrane.

When the pore size of the membrane is about 3 μ m, it somewhat slows the movement of the cells from the top side of the membrane to the bottom side. Motility of the cells is measured over the next several consecutive days. The rate of motility of a given cell line can be determined quantitatively by counting the cell number at the bottom of the membrane after trypsinization. Using this *in vitro* method, the motility rates of PC-3 and PC-huMUC18 human prostate cancer cells, with and without an over-expression of the human MUC18 protein, respectively, are determined and compared.

For the invasiveness of the prostate cancer cells, a similar kind of chamber is used, except before seeding the cells to the top well, the polycarbonate membrane is pre-coated with matrigel that contains protein components of the basal membranes of blood vessels. When the concentration of matrigel is correct, the membrane thus formed is thick enough to form a barrier to stop the cells from penetrating immediately, but is thin enough to allow cells to gradually invade through the membrane and migrate to the bottom of the membrane. Matrigel, which contains protein components of the base membrane of blood cell membrane, such as laminin, collagen type IV, entactin (nedogen), and heparin sulfate proteoglycan, is available commercially through Collaborative Research (Bedford, MA). Each filter in each 6.5 mm well is coated with 100 μ l of a 1:20 dilution of commercial Matrigel in cold DMEM

(about 30 µg per filter). Using a similar method of counting the cells, which grow and attach to the bottom of the membrane, the rate of invasiveness of a given cell line can be quantitated [Repesh, L.A. (1989) *supra*]. To count the cells at the bottom of the membrane, the bottom of the membrane is treated with trypsin to detach the cells, and the cell number is counted
5 directly using a Hemacytometer, or the cells on the membrane is stained with trypan blue and counted directly using a microscope. Alternatively, the cells are labeled with 0.6 µCi of ¹²⁵I-iododeoxyuridine (5 Ci/mg) for 18-24 h (about 95% of cells) and seeded to the top of the transwell chamber. After 72 h of invasion, the cells at the bottom and the cells on the top are trypsinized. The total input radioactivity is determined and compared to the radioactivities
10 associated with cells from the top and bottom chambers. In this way the percentage of cells invading through the membrane can be accurately quantitated. Invasion rate can thus be determined [Repesh, L.A. (1989) *Invasion & Metast.* 9:192-208]. This type of *in vitro* test has been demonstrated to produce results in agreement with that of the *in vivo* animal tests [Repesh, L.A. (1989) *supra*]. Using this *in vitro* method, the invasion rates of LNCAP and LNCAP-huMUC18 transformed or transfected to express MUC18 human prostate cells, with and without over-expression of the human MUC18 protein, respectively, are determined and compared.

20 For comparison, the metastasis rates of these prostate cells are also tested *in vivo* in athymic nude mice [van Weerden et al. (1996) *Am. J. Pathol.* 149, 1055-1062]. The effect of the different expression levels of human MUC18 in prostate cancer cells on metastatic ability of different clones is determined. Similar pairs of cells as used for *in vitro* assay are also used in animal tests. The cells are implanted subcutaneously into nude mice. The size of tumors after different times are measured. The time and extent of the tumor cell metastases to bone or to other organs are investigated [van Weerden et al. (1996) *supra*].

25 The experiments set forth above confirm that the relative level of MUC18 expression in prostate cancer cells correlates positively with metastatic ability.

Example 3. Transcriptional Expression of MUC18

30 Total cell RNA is prepared from cell lines or from prostate tumor tissue with the method of one step acid-guanidinium-thiocyanate-phenol-chloroform extraction [Chomczyuski and Sacchi (1987) *Anal. Biochem.* 162, 156-159]. We have shown that this

method consistently yields good quality RNA for RT-PCR. Poly(A)+RNA is prepared from total RNA by purifying through an oligo(dT)-cellulose column [Aviv and Leder (1972) *J. Molec. Biol.* 134, 743; Wu et al. (1985) *Int. J. Biochem.* 17, 355-363].

5 Northern hybridization technology is used to determine MUC18 mRNA levels expressed in the four prostate cancer cell lines [Ausubel et al. (1987) *Current Protocols in Molecular Biology*, Section 4]. Total RNA is extracted from cell preparations, electrophoretically separated in a formaldehyde-impregnated agarose gel, blotted and hybridized to a ³²P-labeled human MUC18 cDNA [Feinberg and Vogelstein (1983) *Anal. Biochem.* 132, 6-13]. Relative levels of MUC18 mRNA are detected by radioautography.

10 The amount of RNA loaded and transferred is estimated by the intensities of the 28S and 16S bands in each lane. Alternately, G3PDH mRNA can be estimated using a G3PDH-specific probe or 28S and 18S probes after stripping the MUC18-specific probe.

15 Quantitative RT-PCR methodology [Innis et al. (1990) *PCR Protocols*, Academic Press; Quantitative RT-PCR (1993) Methods and Applications Book #3, Clontech, Palo Alto, CA]. Human Sk-Mel-28 cells are used as positive control for this method.

20 Because the quantity of mRNA from a small amount of tissue is small, quantitative RT-PCR is used for quantifying MUC18 mRNA expression in tissues [Innis et al. (1990) *supra*]. The isolation kit from Boehringer-Mannheim (Indianapolis, IN) using magnetic beads is suitable for obtaining a small amount of poly(A)+RNA from prostate tissue. The quality of mRNA isolated with this kit is also excellent for translation and RT-PCR. The quantitative RT-PCR method is first established from using mRNA of cultured cells, as described above, and then is used for quantifying the expression of MUC18 mRNA in different prostate cancer tissues.

25 The RT step is standard: a 20 μ l RT reaction contains 1 μ g of poly(A)+RNA from the human melanoma cell line, SK-Mel-28, as template, 0.5 μ g of oligo(dT)₁₆ as primer (Promega), 2 μ l of 10X AMV-RT buffer (Promega, Madison, WI), 2 units of AMV-reverse transcriptase, 5 mM MgCl₂, 1 mM of dNTP mix (Promega), 1 unit of RNase inhibitor (Promega), and 50 μ g/ml of acetyl BSA. The reaction was carried out at 42 to 48 °C for one hour, and heated at 99 °C for 5 min.

30 A 20 μ l PCR reaction contained 2 μ l of RT reaction mixture (containing the first-strain cDNA), 2 μ l each of the two primers (20 pm/ μ l), 0.01 mM dNTPs, 1 μ g of acetyl BSA,

2 μ l of 10X PCR buffer with 15 mM MgCl₂ (Promega), and 0.5 units of Taq DNA polymerase (Promega, 5 units/ μ l). PCR cycles are as follows:

Hot start at 94°C 5 min, 80°C 30 sec
29 cycles of 94°C 30 sec, 64-66°C 30 sec, and 72°C 2 min
1 cycle of 94°C 30 sec, 64-66°C 30 sec, and 72°C 60 min

5

The sequences of the primers for amplification of the human MUC18 cDNA from poly(A)+RNA of human melanoma cell line SK-Mel-28 are as follows:

BF1 27-mer 5'-CTCGGGATCCATGGGGCTTCCCAGGCT (SEQ ID NO:9)
ER6A 25-mer 5'-TCGGGGCTAATGCCTCAGATCGATG (SEQ ID NO:10)

10

Example 4. Immunofluorescence Assay for MUC18

Where the anti-human MUC18 antibodies are made in chicken, fluorescence-tagged anti-chicken IgG are used for immunofluorescent staining. Tissue culture cells, or normal or cancer prostate tissue samples are fixed, and first reacted with the anti-human MUC18 antibodies, washed, and then reacted with the fluorescence-tagged rabbit anti-chicken antibodies [Umbas et al. (1992) *Canc. Res.* 52, 5104-5109]. The presence of human MUC18 on the surface of these cells or tissues readily detected using UV-fluorescence microscopy.

Human melanoma cells, Sk-Mel-28 (ATCC HTB 72), which express MUC18 a high level of MUC18 are used as the positive control. Human melanoma cells, WM115 (ATCC CRL 1675) which express no MUC18, are used as the negative control. Three human prostate cancer cell lines, LNCap.FGC, PC-3, and DU145 are available from American Type Culture Collection, Rockville, MD, as ATCC CRL 1740, ATCC CRL 1435 and ATCC HTB 81, respectively. One other cell line, TSU-PR1, isolated by Dr. Iizumi in Japan [Iizumi et al. (1987) *J. Urol.* 137, *J. Urol.* 137:1304-1306] and provided by Dr. John T. Isaacs, John Hopkins University, Baltimore, MD] was tested. TSU-PR1 cells are more metastatic than the other three cell lines [Graff et al. (1995) *Cancer Res.* 55, 5195-5199].

All four cell lines are grown as monolayer cultures in a 37°C incubator with an atmosphere of 5% CO₂. Tsu-PR1 and LNCap.FGC cells are grown in RPMI 1640 supplemented with 10% fetal bovine serum. PC-cells are grown in F12K medium with 10% fetal bovine serum. DU145 cells are grown in EMEM medium supplemented with pyruvate, extra non-essential amino acids and vitamins, and 10% fetal bovine serum.

Biopsy samples are taken, fixed and subjected to immunoassay using polyclonal antibody specific for human MUC18 described hereinabove, as described in Wood et al. (1994) *Cancer* 74:2533-2540.

1 CLAIM:

1. A method for identifying metastatic potential of a prostate cancer cell, said method comprising the step of:

5 detecting expression of a MUC18 coding sequence in a prostate cancer cell for which an identification of metastatic potential is sought relative to expression of a MUC18 coding sequence in a normal prostate cell, wherein a higher level of expression of the MUC18 coding sequence is positively correlated with metastatic potential of a prostate cancer cell,

10 whereby metastatic potential of a prostate cancer cell is deemed high when the level of expression of a MUC18 coding sequence is higher in said prostate cancer cell than in a normal prostate cell.

2. The method of claim 1, wherein said prostate cancer cell is from a biopsy tissue sample from a patient for whom a prediction of metastasis of prostate cancer is sought.

3. The method of claim 1, wherein expression of MUC18 coding sequence is determined by immunoassay.

4. The method of claim 3, wherein expression of the MUC18 coding sequence is determined by immunoassay using antibody made in an experimental laboratory animal in response to a MUC 18 antigen.

20 5. The method of claim 4, wherein the MUC18 antigen is a middle portion of MUC18.

6. The method of claim 5, wherein said middle portion of MUC18 has an amino acid sequence as given in SEQ ID NO:2, amino acids 211-376.

7. The method of claim 1, wherein expression of a MUC18 coding sequence is determined by Northern hybridization.

8. The method of claim 7, wherein a probe used in Northern hybridization comprises at least 15 contiguous nucleotides of SEQ ID NO:1.

5 9. The method of claim 8, wherein a probe used in Northern hybridization comprises a nucleotide sequence as given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:10.

10. The method of claim 1, wherein said expression of a MUC18 coding sequence is determined by a reverse transcriptase-polymerase chain reaction.

10 11. The method of claim 10, wherein a primer used in the reverse-transcriptase polymerase chain reaction comprises a nucleotide sequence as given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9 or SEQ ID NO:10.

12. The method of claim 1, wherein said prostate cancer cell is a cell line cell.

13. An immunoassay kit for diagnosing metastatic potential of a prostate cancer cell, said kit comprising an antibody made in response to immunization with an antigen consisting essentially of middle portion MUC18.

14. A nucleic acid vector comprising a nucleotide sequence encoding a middle portion MUC18 protein, said middle portion MUC18 protein consisting essentially of an amino acid sequence as given in SEQ ID NO:2, amino acids 211-376.

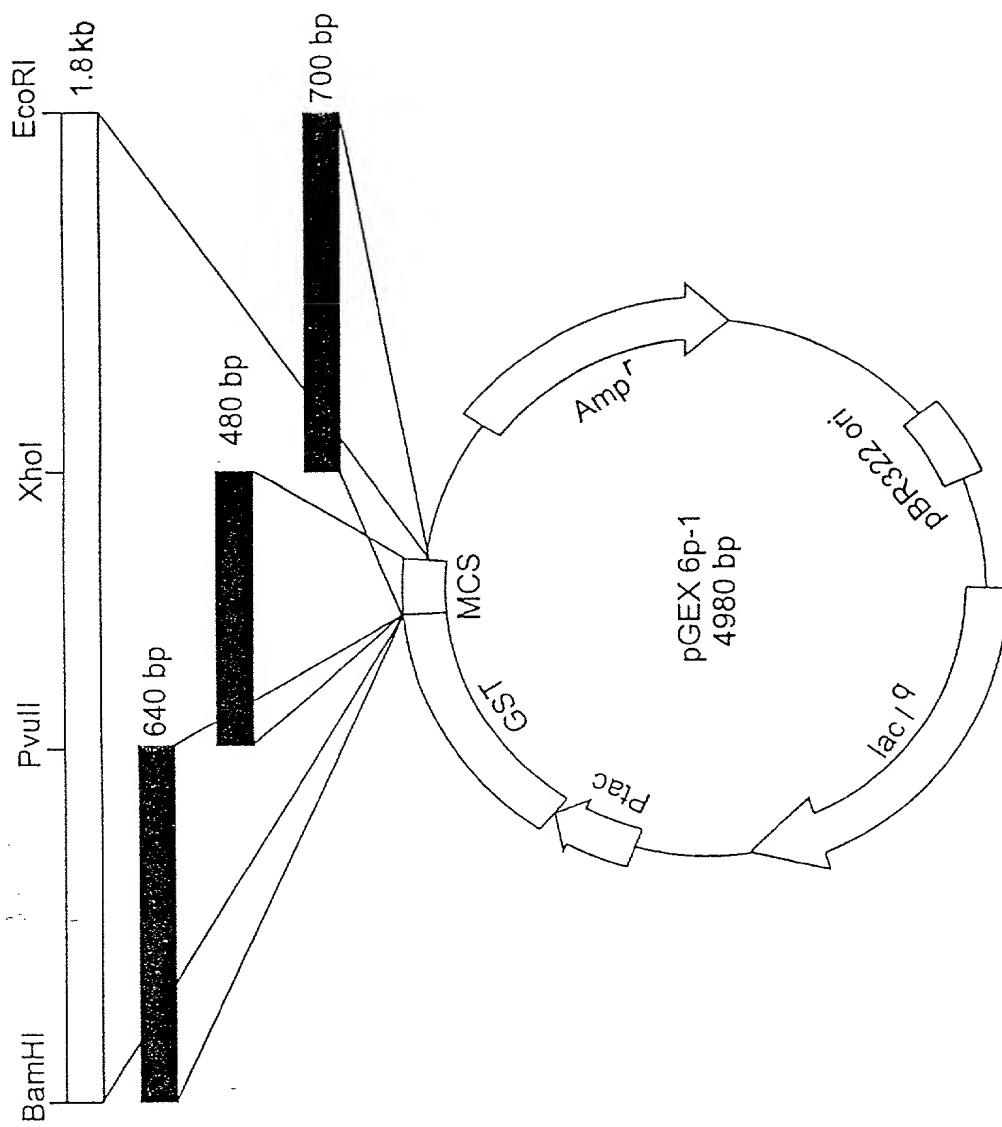
20 15. The nucleic acid vector of claim 14, wherein said vector comprises a nucleotide sequence encoding a middle portion MUC18 protein as given in SEQ ID NO:1, nucleotides 631-1128.

16. A recombinant host cell comprising the nucleic acid vector of claim 14.
17. A nucleic acid vector comprising a nucleotide sequence encoding a MUC18 protein, said MUC18 protein being characterized by an amino acid sequence as given in SEQ ID NO:2.
- 5 18. The nucleic acid vector of claim 17, wherein said nucleotide sequence encoding a MUC18 protein is as given in SEQ ID NO:1, nucleotides 1-1938.
19. A recombinant host cell comprising the nucleic acid vector of claim 17.

ABSTRACT

The present disclosure provides methods for the diagnosis of metastatic prostate cancer and/or the prediction of the metastatic ability of prostate cancer in prostate biopsy tissue. Metastatic ability of prostate cancer is positively correlated with the level of transcriptional and translational expression of the MUC18 coding sequence in the neoplastic tissue. Methods for the determination of MUC18 protein synthesis include Western blots, ELISA, radioimmunoassay, immunofluorescence, and other immunoassays using MUC18-specific antibody and suitable detection means. Methods for measurement of transcriptional expression of the MUC18 coding sequence include Northern hybridizations and quantitative reverse transcriptase-polymerase chain reaction analyses. Absence of or very low MUC18 expression in the prostate tumor tissue is associated with nonmetastatic cancer, while relatively high levels of MUC18 expression are predictive of prostate cancer which is likely to metastasize or which has already metastasized. The present disclosure provides an improved diagnostic tool to aid the medical community in the choice of appropriate treatment regimens for prostate cancer patients.

15



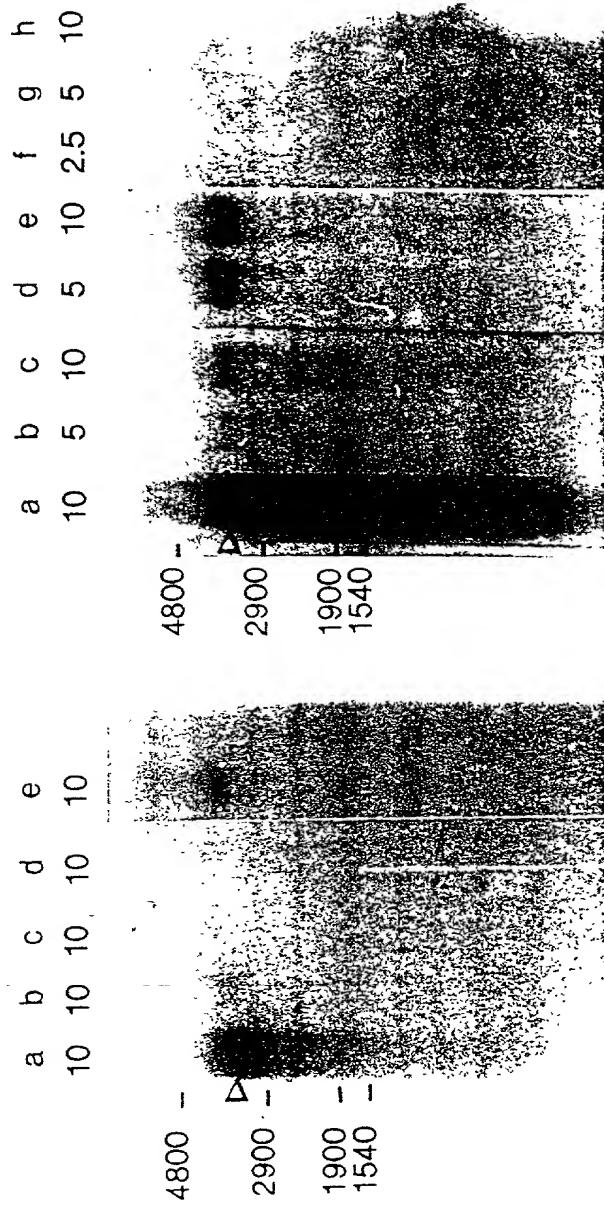
m1m2 a



2036-1636-

三

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SK, M. PC-3, DU
SK, DU, TSUPRI, LNCAP

FIG. 2A
FIG. 2B

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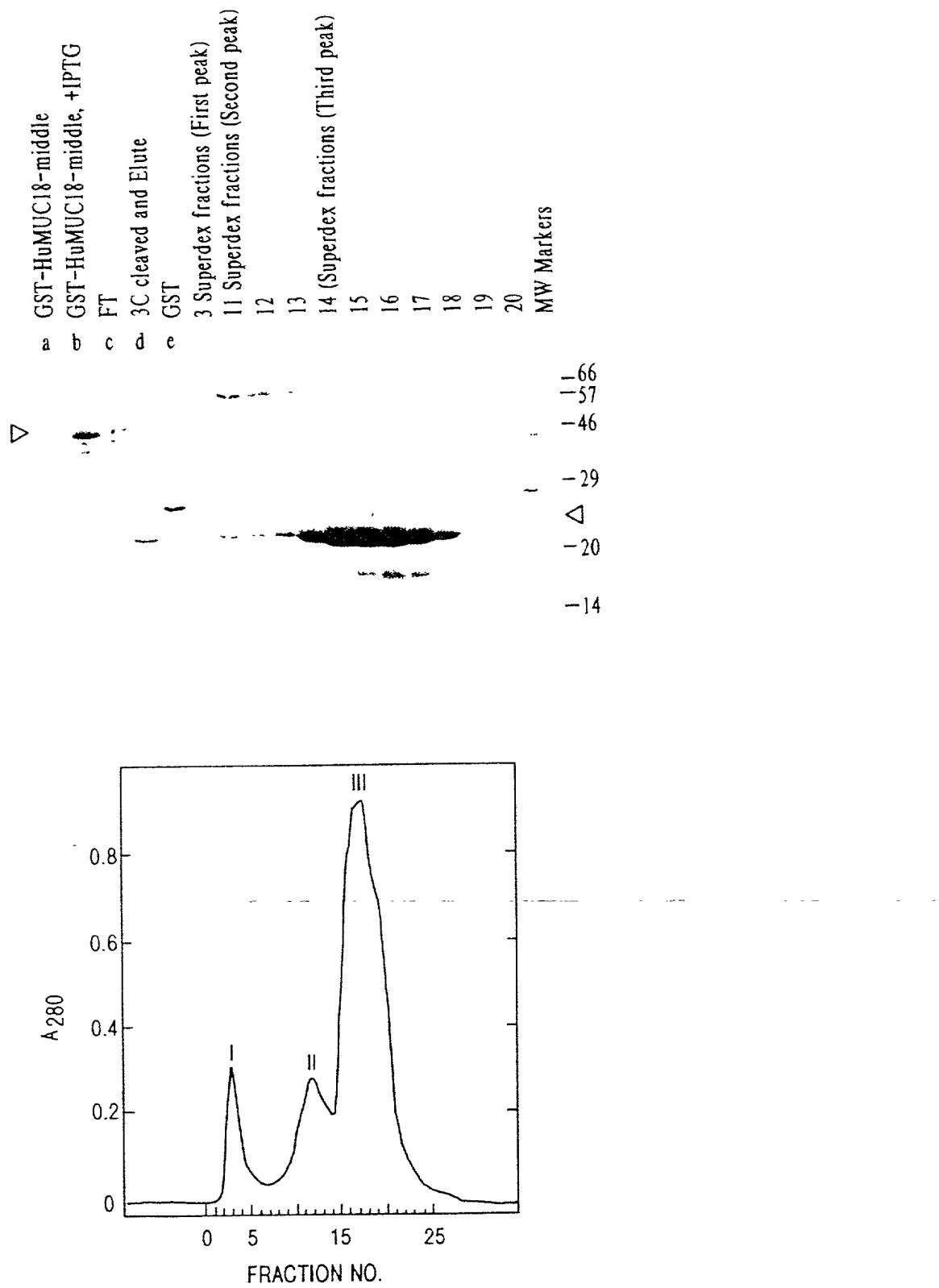


FIG. 3

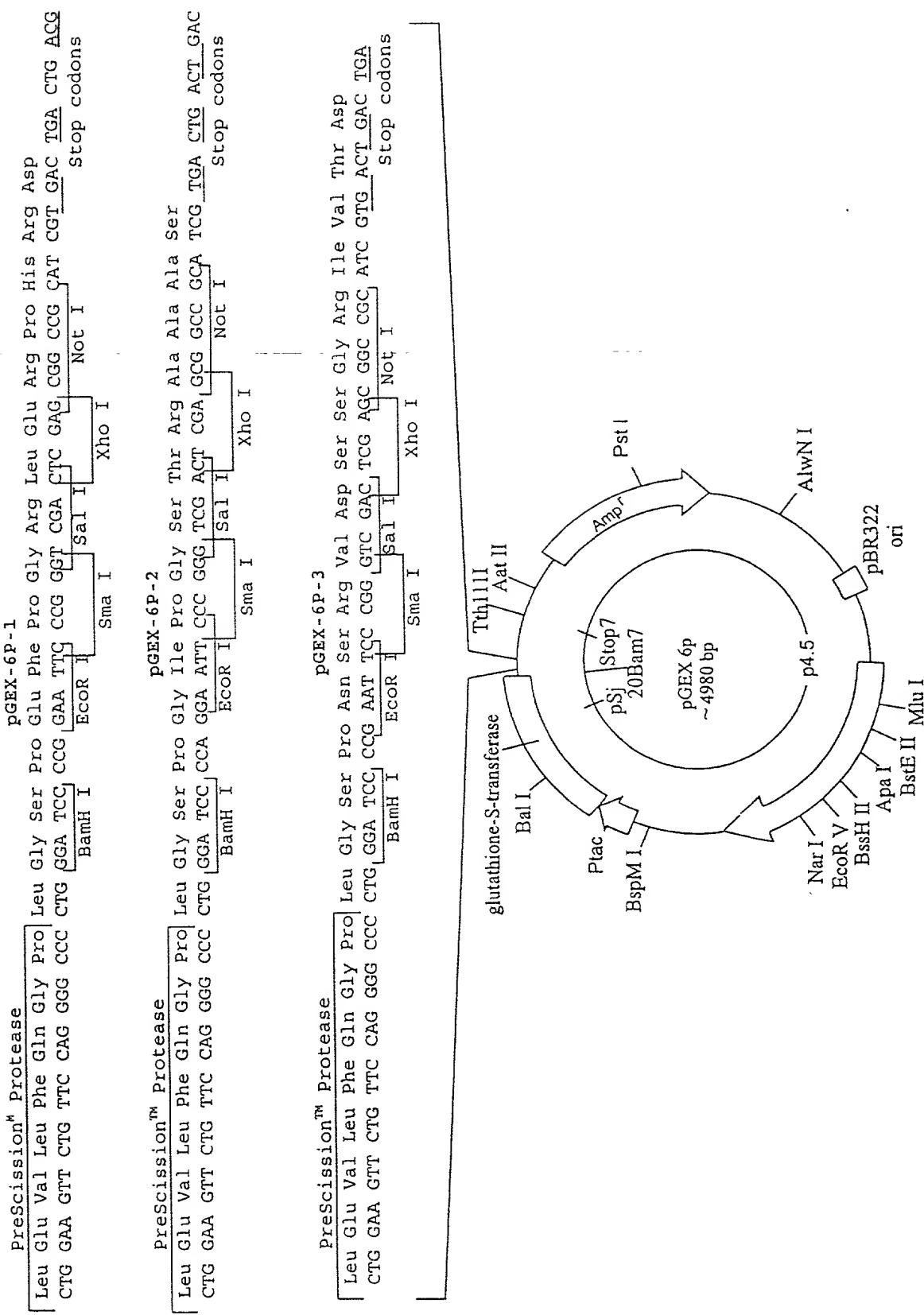
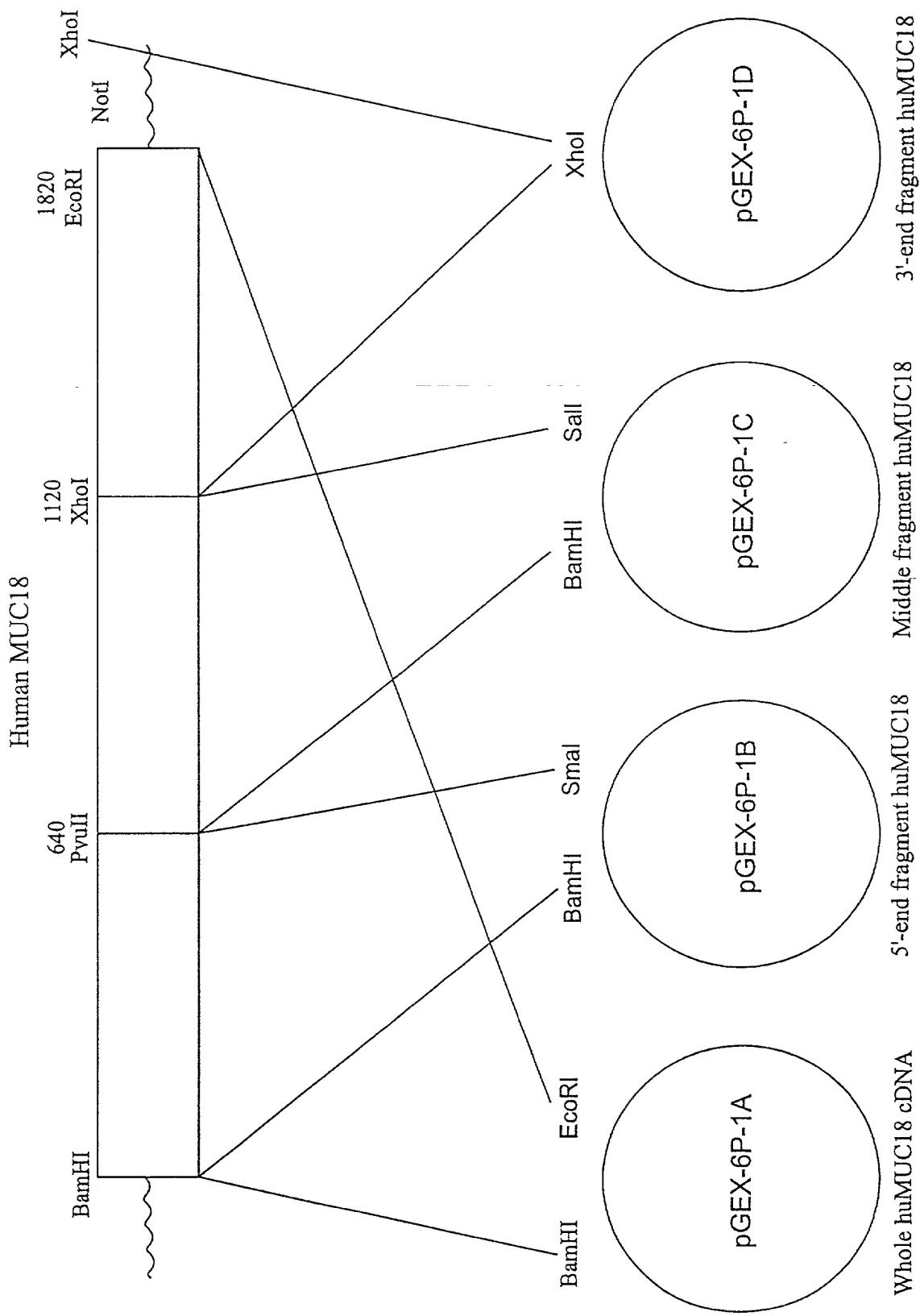


FIG. 4



5

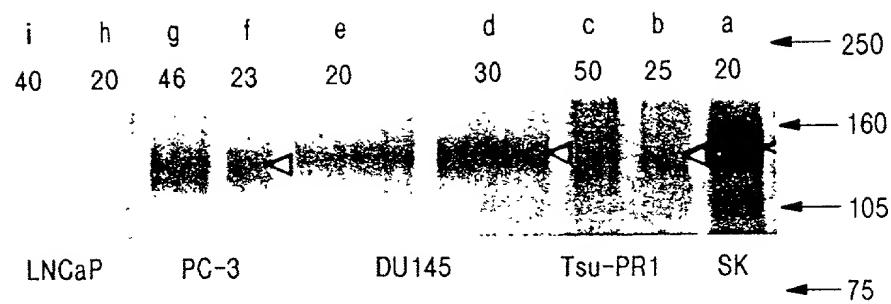


FIG. 6

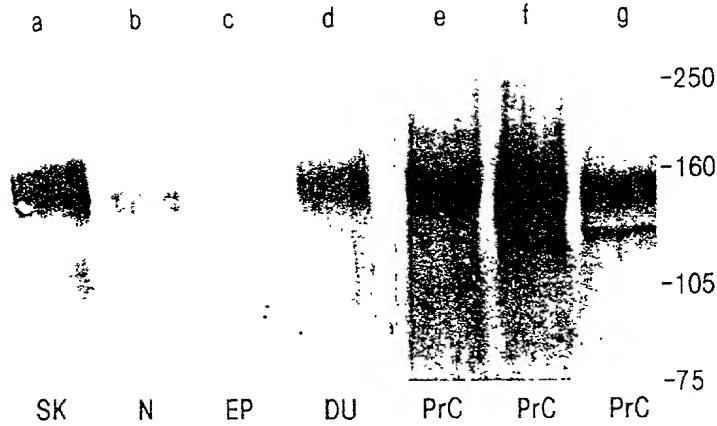


FIG. 7

**INVENTOR'S DECLARATION FOR PATENT APPLICATION
AND POWER OF ATTORNEY**

As the below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name;

I believe that I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DIAGNOSTIC FOR METASTATIC PROSTATE CANCER,

the specification of which:

X is attached hereto.

— was filed on _____ as

and was amended on N/A (if applicable).

I hereby authorize our legal representative to add reference to the Serial No. and/or filing date of the above-referenced application to this declaration.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

Prior Foreign Application(s)

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

Country	Application No.	Date of Filing (day,month,year)	Date of Issue (day,month,year)	Priority Claimed
NONE				35 U.S.C.119
				Yes <u> </u> No <u> </u>

Prior Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial Number	Date of Filing
60/076,664	3 March 1998

Prior U.S. Application(s) and PCT International Application(s) Designating the United States

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States listed below:

Application Serial Number	Date of Filing	Status
PCT US99/04850	2 March 1999	Pending

Insofar as the subject matter of each of the claims in this application is not disclosed in the prior United States, foreign or PCT International application(s) to which priority has been claimed above in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby appoint, both jointly and severally, as our attorneys and agents with full power of substitution and revocation, to prosecute this application and any corresponding application filed in the Patent Cooperation Treaty Receiving Office, and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

Lorance L. Greenlee, Reg. No. 27,894; Ellen P. Winner, Reg. No. 28,547; Sally A. Sullivan, Reg. No. 32,064; Donna M. Ferber, Reg. No. 33,878; G. William Van Cleave, Reg. No. 40,213; Susan K. Doughty, Reg. No. 43,595; and Heeja Yoo-Warren, Reg. No. 45,495, all of Greenlee, Winner and Sullivan, P.C., 5370 Manhattan Circle, Suite 201, Boulder, CO 80303.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Date 8/31/00

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ccc ctg ggc atc cct gtg aac agt aag gag cct gag gag gtc gct acc Pro Leu Gly Ile Pro Val Asn Ser Lys Glu Pro Glu Glu Val Ala Thr	145	150	155	160	480
tgt gta ggg agg aac ggg tac ccc att cct caa gtc atc tgg tac aag Cys Val Gly Arg Asn Gly Tyr Pro Ile Pro Gln Val Ile Trp Tyr Lys	165	170	175	528	
aat ggc cgg cct ctg aag gag gag aag aac ccg gtc cac att cag tcg Asn Gly Arg Pro Leu Lys Glu Glu Lys Asn Arg Val His Ile Gln Ser	180	185	190	576	
tcc cag act gtg gag tcg agt ggt ttg tac acc ttg cag agt att ctg Ser Gln Thr Val Glu Ser Ser Gly Leu Tyr Thr Leu Gln Ser Ile Leu	195	200	205	624	
aag gca cag ctg gtt aaa gaa gac aaa gat gcc cag ttt tac tgt gag Lys Ala Gln Leu Val Lys Glu Asp Lys Asp Ala Gln Phe Tyr Cys Glu	210	215	220	672	
ctc aac tac ccg ctg ccc agt ggg aac cac atg aag gag tcc agg gaa Leu Asn Tyr Arg Leu Pro Ser Gly Asn His Met Lys Glu Ser Arg Glu	225	230	235	240	720
gtc acc gtc cct gtt ttc tac ccg aca gaa aaa gtg tgg ctg gaa gtg Val Thr Val Pro Val Phe Tyr Pro Thr Glu Lys Val Trp Leu Glu Val	245	250	255	768	
gag ccc gtg gga atg ctg aag gaa ggg gac cg ^c gtg gaa atc agg tgt Glu Pro Val Gly Met Leu Lys Glu Gly Asp Arg Val Glu Ile Arg Cys	260	265	270	816	
ttg gct gat ggc aac cct cca cca cac ttc agc atc agc aag cag aac Leu Ala Asp Gly Asn Pro Pro His Phe Ser Ile Ser Lys Gln Asn	275	280	285	864	

ccc agc acc agg gag gca gag gaa gag aca acc aac gac aac ggg gtc	912		
Pro Ser Thr Arg Glu Ala Glu Glu Thr Thr Asn Asp Asn Gly Val			
290	295	300	
ctg gtg ctg gag cct gcc cgg aag gaa cac agt ggg cgc tat gaa tgt	960		
Leu Val Leu Glu Pro Ala Arg Lys Glu His Ser Gly Arg Tyr Glu Cys			
305	310	315	320
cag ggc ctg gac ttg gac acc atg ata tcg ctg agt gaa cca cag	1008		
Gln Gly Leu Asp Leu Asp Thr Met Ile Ser Leu Leu Ser Glu Pro Gln			
325	330	335	
gaa cta ctg gtg aac tat gtg tct gac gtc cga gtg agt ccc gca gcc	1056		
Glu Leu Leu Val Asn Tyr Val Ser Asp Val Arg Val Ser Pro Ala Ala			
340	345	350	
cct gag aga cag gaa ggc agc agc ctc acc ctg acc tgt gag gca gag	1104		
Pro Glu Arg Gln Glu Gly Ser Ser Leu Thr Leu Thr Cys Glu Ala Glu			
355	360	365	
agt agc cag gac ctc gag ttc cag tgg ctg aga gaa gag aca ggc cag	1152		
Ser Ser Gln Asp Leu Glu Phe Gln Trp Leu Arg Glu Glu Thr Gly Gln			
370	375	380	
gtg ctg gaa agg ggg cct gtg ctt cag ttg cat gac ctg aaa cgg gag	1200		
Val Leu Glu Arg Gly Pro Val Leu Gln Leu His Asp Leu Lys Arg Glu			
385	390	395	400
gca gga ggc ggc tat cgc tgc gtg gcg tct gtg ccc agc ata ccc gga	1248		
Ala Gly Gly Tyr Arg Cys Val Ala Ser Val Pro Ser Ile Pro Gly			
405	410	415	
ctg aac cgc aca cag ctg gtc aac gtg gcc att ttt ggc ccc cct tgg	1296		
Leu Asn Arg Thr Gln Leu Val Asn Val Ala Ile Phe Gly Pro Pro Trp			
420	425	430	
atg gca ttc aag gag agg aag gtg tgg gtg aaa gag aat atg gtg ttg	1344		
Met Ala Phe Lys Glu Arg Lys Val Trp Val Lys Glu Asn Met Val Leu			
435	440	445	
aat ctg tct tgt gaa gcg tca ggg cac ccc cgg ccc acc atc tcc tgg	1392		
Asn Leu Ser Cys Glu Ala Ser Gly His Pro Arg Pro Thr Ile Ser Trp			
450	455	460	
aac gtc aac ggc acg gca agt gaa caa gac caa gat cca cag cga gtc	1440		
Asn Val Asn Gly Thr Ala Ser Glu Gln Asp Gln Asp Pro Gln Arg Val			
465	470	475	480
ctg agc acc ctg aat gtc ctc gtg acc ccg gag ctg ttg gag aca ggt	1488		
Leu Ser Thr Leu Asn Val Leu Val Thr Pro Glu Leu Leu Glu Thr Gly			
485	490	495	

gtt gaa tgc acg gcc tcc aac gac ctg ggc aaa aac acc acc agc atc ctc		1536	
Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser Ile Leu			
500	505	510	
ttc ctg gag ctg gtc aat tta acc acc ctc aca cca gac tcc aac aca		1584	
Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser Asn Thr			
515	520	525	
acc act ggc ctc agc act tcc act gcc agt cct cat acc acc aga gcc aac		1632	
Thr Thr Gly Leu Ser Thr Ser Ala Ser Pro His Thr Arg Ala Asn			
530	535	540	
agc acc tcc aca gag aga aag ctg ccg gag ccg gag agc cgg ggc gtg		1680	
Ser Thr Ser Thr Glu Arg Lys Leu Pro Glu Pro Glu Ser Arg Gly Val			
545	550	555	560
gtc atc gtg gct gtg att gtg tgc atc ctg gtc ctg gcg gtg ctg ggc		1728	
Val Ile Val Ala Val Ile Val Cys Ile Leu Val Leu Ala Val Leu Gly			
565	570	575	
gct gtc ctc tat ttc ctc tat aag aag ggc aag ctg ccg tgc agg cgc		1776	
Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys Arg Arg			
580	585	590	
tca ggg aag cag gag atc acg ctg ccc ccg tct cgt aag agc gaa ctt		1824	
Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Ser Glu Leu			
595	600	605	
gta gtt gaa gtt aag tca gat aag ctc cca gaa gag atg ggc ctc ctg		1872	
Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly Leu Leu			
610	615	620	
cag ggc agc agc ggt gac aag agg gct ccg gga gac cag gga gag aaa		1920	
Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly Glu Lys			
625	630	635	640
tac atc gat ctg agg cat tagccccgaa at		1950	
Tyr Ile Asp Leu Arg His			
645			

<210> 2
<211> 646
<212> PRT
<213> Homo sapiens

<400> 2			
Met Gly Leu Pro Arg Leu Val Cys Ala Phe Leu Leu Ala Ala Cys Cys			
1	5	10	15
Cys Cys Pro Arg Val Ala Gly Val Pro Gly Glu Ala Glu Gln Pro Ala			
20	25	30	

Pro	Glu	Leu	Val	Glu	Val	Glu	Val	Gly	Ser	Thr	Ala	Leu	Leu	Lys	Cys		
35														45			
Gly	Leu	Ser	Gln	Ser	Gln	Gly	Asn	Leu	Ser	His	Val	Asp	Trp	Phe	Ser		
50														55	60		
Val	His	Lys	Glu	Lys	Arg	Thr	Leu	Ile	Phe	Arg	Val	Arg	Gln	Gly	Gln		
65														75	80		
Gly	Gln	Ser	Glu	Pro	Gly	Glu	Tyr	Glu	Gln	Arg	Leu	Ser	Leu	Gln	Asp		
85														90	95		
Arg	Gly	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Val	Thr	Pro	Gln	Asp	Glu	Arg		
100														105	110		
Ile	Phe	Leu	Cys	Gln	Gly	Lys	Arg	Pro	Arg	Ser	Gln	Glu	Tyr	Arg	Ile		
115														120	125		
Gln	Leu	Arg	Val	Tyr	Lys	Ala	Pro	Glu	Glu	Pro	Asn	Ile	Gln	Val	Asn		
130														135	140		
Pro	Leu	Gly	Ile	Pro	Val	Asn	Ser	Lys	Glu	Pro	Glu	Glu	Val	Ala	Thr		
145														155	160		
Cys	Val	Gly	Arg	Asn	Gly	Tyr	Pro	Ile	Pro	Gln	Val	Ile	Trp	Tyr	Lys		
														165	170	175	
Asn	Gly	Arg	Pro	Leu	Lys	Glu	Glu	Lys	Asn	Arg	Val	His	Ile	Gln	Ser		
														180	185	190	
Ser	Gln	Thr	Val	Glu	Ser	Ser	Gly	Leu	Tyr	Thr	Leu	Gln	Ser	Ile	Leu		
														195	200	205	
Lys	Ala	Gln	Leu	Val	Lys	Glu	Asp	Lys	Asp	Ala	Gln	Phe	Tyr	Cys	Glu		
														210	215	220	
Leu	Asn	Tyr	Arg	Leu	Pro	Ser	Gly	Asn	His	Met	Lys	Glu	Ser	Arg	Glu		
														225	230	235	240
Val	Thr	Val	Pro	Val	Phe	Tyr	Pro	Thr	Glu	Lys	Val	Trp	Leu	Glu	Val		
														245	250	255	
Glu	Pro	Val	Gly	Met	Leu	Lys	Glu	Gly	Asp	Arg	Val	Glu	Ile	Arg	Cys		
														260	265	270	
Leu	Ala	Asp	Gly	Asn	Pro	Pro	Pro	His	Phe	Ser	Ile	Ser	Lys	Gln	Asn		
														275	280	285	
Pro	Ser	Thr	Arg	Glu	Ala	Glu	Glu	Thr	Thr	Asn	Asp	Asn	Gly	Val			
														290	295	300	

Leu Val Leu Glu Pro Ala Arg Lys Glu His Ser Gly Arg Tyr Glu Cys
305 310 315 320

Gln Gly Leu Asp Leu Asp Thr Met Ile Ser Leu Leu Ser Glu Pro Gln
325 330 335

Glu Leu Leu Val Asn Tyr Val Ser Asp Val Arg Val Ser Pro Ala Ala
340 345 350

Pro Glu Arg Gln Glu Gly Ser Ser Leu Thr Leu Thr Cys Glu Ala Glu
355 360 365

Ser Ser Gln Asp Leu Glu Phe Gln Trp Leu Arg Glu Glu Thr Gly Gln
370 375 380

Val Leu Glu Arg Gly Pro Val Leu Gln Leu His Asp Leu Lys Arg Glu
385 390 395 400

Ala Gly Gly Gly Tyr Arg Cys Val Ala Ser Val Pro Ser Ile Pro Gly
405 410 415

Leu Asn Arg Thr Gln Leu Val Asn Val Ala Ile Phe Gly Pro Pro Trp
420 425 430

Met Ala Phe Lys Glu Arg Lys Val Trp Val Lys Glu Asn Met Val Leu
435 440 445

Asn Leu Ser Cys Glu Ala Ser Gly His Pro Arg Pro Thr Ile Ser Trp
450 455 460

Asn Val Asn Gly Thr Ala Ser Glu Gln Asp Gln Asp Pro Gln Arg Val
465 470 475 480

Leu Ser Thr Leu Asn Val Leu Val Thr Pro Glu Leu Leu Glu Thr Gly
485 490 495

Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser Ile Leu
500 505 510

Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser Asn Thr
515 520 525

Thr Thr Gly Leu Ser Thr Ser Thr Ala Ser Pro His Thr Arg Ala Asn
530 535 540

Ser Thr Ser Thr Glu Arg Lys Leu Pro Glu Pro Glu Ser Arg Gly Val
545 550 555 560

Val Ile Val Ala Val Ile Val Cys Ile Leu Val Leu Ala Val Leu Gly
565 570 575

Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys Arg Arg
 580 585 590
 Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Ser Glu Leu
 595 600 605
 Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly Leu Leu
 610 615 620
 Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly Glu Lys
 625 630 635 640
 Tyr Ile Asp Leu Arg His
 645

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 <213> Homo sapiens

<220>
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 Met Gly Leu Pro Arg Leu Val Cys Ala Phe Leu Leu Ala Ala
 1 5 10
 tgc tgc tgc tgt cct cgc gtc gcg ggt gtg ccc gga gag gct gag cag 97
 Cys Cys Cys Pro Arg Val Ala Gly Val Pro Gly Glu Ala Glu Gln
 15 20 25 30
 cct gcg cct gag ctg gtg gag gtg gaa gtg ggc agc aca gcc ctt ctg 145
 Pro Ala Pro Glu Leu Val Glu Val Gly Ser Thr Ala Leu Leu
 35 40 45
 aag tgc ggc ctc tcc cag tcc caa ggc aac ctc agc cat gtc gac tgg 193
 Lys Cys Gly Leu Ser Gln Ser Gln Gly Asn Leu Ser His Val Asp Trp
 50 55 60
 ttt tct gtc cac aag gag aag cgg acg ctc atc ttc cgt gtg cgc cag 241
 Phe Ser Val His Lys Glu Lys Arg Thr Leu Ile Phe Arg Val Arg Gln
 65 70 75
 ggc cag ggc cag agc gaa cct ggg gag tac gag cag cgg ctc agc ctc 289
 Gly Gln Gly Gln Ser Glu Pro Gly Glu Tyr Glu Gln Arg Leu Ser Leu
 80 85 90

cag gac aga ggg gct act ctg gcc ctg act caa gtc acc ccc caa gac Gln Asp Arg Gly Ala Thr Leu Ala Leu Thr Gln Val Thr Pro Gln Asp 95	100	105	110	337
gag cgc atc ttc ttg tgc cag ggc aag cgc cct cgg tcc cag gag tac Glu Arg Ile Phe Leu Cys Gln Gly Lys Arg Pro Arg Ser Gln Glu Tyr 115	120	125		385
cgc atc cag ctc cgc gtc tac aaa gct ccg gag gag cca aac atc cag Arg Ile Gln Leu Arg Val Tyr Lys Ala Pro Glu Glu Pro Asn Ile Gln 130	135	140		433
gtc aac ccc ctg ggc atc cct gtg aac agt aag gag cct gag gag gtc Val Asn Pro Leu Gly Ile Pro Val Asn Ser Lys Glu Pro Glu Glu Val 145	150	155		481
gct acc tgt gta ggg agg aac ggg tac ccc att cct caa gtc atc tgg Ala Thr Cys Val Gly Arg Asn Gly Tyr Pro Ile Pro Gln Val Ile Trp 160	165	170		529
tac aag aat ggc cgg cct ctg aag gag gag aag aac cgg gtc cac att Tyr Lys Asn Gly Arg Pro Leu Lys Glu Glu Lys Asn Arg Val His Ile 175	180	185	190	577
cag tcg tcc cag act gtg gag tcg agt ggt ttg tac acc ttg cag agt Gln Ser Ser Gln Thr Val Glu Ser Ser Gly Leu Tyr Thr Leu Gln Ser 195	200	205		625
att ctg aag gca cag ctg gtt aaa gaa gac aaa gat gcc cag ttt tac Ile Leu Lys Ala Gln Leu Val Lys Glu Asp Lys Asp Ala Gln Phe Tyr 210	215	220		673
tgt gag ctc aac tac cgg ctg ccc agt ggg aac cac atg aag gag tcc Cys Glu Leu Asn Tyr Arg Leu Pro Ser Gly Asn His Met Lys Glu Ser 225	230	235		721
agg gaa gtc acc gtc cct gtt ttc tac ccg aca gaa aaa gtg tgg ctg Arg Glu Val Thr Val Pro Val Phe Tyr Pro Thr Glu Lys Val Trp Leu 240	245	250		769
gaa gtg gag ccc gtg gga atg ctg aag gaa ggg gac cgc gtg gaa atc Glu Val Glu Pro Val Gly Met Leu Lys Glu Gly Asp Arg Val Glu Ile 255	260	265	270	817
agg tgt ttg gct gat ggc aac cct cca cca cac ttc agc atc agc aag Arg Cys Leu Ala Asp Gly Asn Pro Pro His Phe Ser Ile Ser Lys 275	280	285		865
cag aac ccc agc acc agg gag gca gag gaa gag aca acc aac gac aac Gln Asn Pro Ser Thr Arg Glu Ala Glu Glu Thr Thr Asn Asp Asn 290	295	300		913

ggg gtc ctg gtg ctg gag cct gcc cg ^g aag gaa cac agt ggg cgc tat		961
Gly Val Leu Val Leu Glu Pro Ala Arg Lys Glu His Ser Gly Arg Tyr		
305	310	315
gaa tgt cag gcc tgg aac ttg gac acc atg ata tcg ctg ctg agt gaa		1009
Glu Cys Gln Ala Trp Asn Leu Asp Thr Met Ile Ser Leu Leu Ser Glu		
320	325	330
cca cag gaa cta ctg gtg aac tat gtg tct gac gtc cga gtg agt ccc		1057
Pro Gln Glu Leu Leu Val Asn Tyr Val Ser Asp Val Arg Val Ser Pro		
335	340	345
350		
gca gcc cct gag aga cag gaa ggc agc agc ctc acc ctg acc tgt gag		1105
Ala Ala Pro Glu Arg Gln Glu Gly Ser Ser Leu Thr Leu Thr Cys Glu		
355	360	365
gca gag agt agc cag gac ctc gag ttc cag tgg ctg aga gaa gag aca		1153
Ala Glu Ser Ser Gln Asp Leu Glu Phe Gln Trp Leu Arg Glu Glu Thr		
370	375	380
gac cag gtg ctg gaa agg ggg cct gtg ctt cag ttg cat gac ctg aaa		1201
Asp Gln Val Leu Glu Arg Gly Pro Val Leu Gln Leu His Asp Leu Lys		
385	390	395
cg ^g gag gca gga ggc ggc tat cgc tgc gtg gcg tct gtg ccc agc ata		1249
Arg Glu Ala Gly Gly Tyr Arg Cys Val Ala Ser Val Pro Ser Ile		
400	405	410
ccc ggc ctg aac cgc aca cag ctg gtc aag ctg gcc att ttt ggc ccc		1297
Pro Gly Leu Asn Arg Thr Gln Leu Val Lys Leu Ala Ile Phe Gly Pro		
415	420	425
430		
cct tgg atg gca ttc aag gag agg aag gtg tgg gtg aaa gag aat atg		1345
Pro Trp Met Ala Phe Lys Glu Arg Lys Val Trp Val Lys Glu Asn Met		
435	440	445
gtg ttg aat ctg tct tgt gaa gcg tca ggg cac ccc cgg ccc acc atc		1393
Val Leu Asn Leu Ser Cys Glu Ala Ser Gly His Pro Arg Pro Thr Ile		
450	455	460
tcc tgg aac gtc aac ggc acg gca agt gaa caa gac caa gat cca cag		1441
Ser Trp Asn Val Asn Gly Thr Ala Ser Glu Gln Asp Gln Asp Pro Gln		
465	470	475
cga gtc ctg agc acc ctg aat gtc ctc gtg acc ccg gag ctg ttg gag		1489
Arg Val Leu Ser Thr Leu Asn Val Leu Val Thr Pro Glu Leu Leu Glu		
480	485	490
aca ggt gtt gaa tgc acg gcc tcc aac gac ctg ggc aaa aac acc agc		1537
Thr Gly Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser		
495	500	505
510		

atc ctc ttc ctg gag ctg gtc aat tta acc acc ctc aca cca gac tcc Ile Leu Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser 515	520	525	1585	
aac aca acc act ggc ctc agc act tcc act gcc agt cct cat acc aga Asn Thr Thr Thr Gly Leu Ser Thr Ser Thr Ala Ser Pro His Thr Arg 530	535	540	1633	
gcc aac agc acc tcc aca gag aga aag ctg ccg gag ccg gag agc cgg Ala Asn Ser Thr Ser Thr Glu Arg Lys Leu Pro Glu Pro Glu Ser Arg 545	550	555	1681	
ggc gtg gtc atc gtg gct gtg att gtg tgc atc ctg gtc ctg gcg gtg Gly Val Val Ile Val Ala Val Ile Val Cys Ile Leu Val Leu Ala Val 560	565	570	1729	
ctg ggc gct gtc ctc tat ttc ctc tat aag aag ggc aag ctg ccg tgc Leu Gly Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys 575	580	585	590	1777
agg cgc tca ggg aag cag gag atc acg ctg ccc ccg tct cgt aag acc Arg Arg Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Thr 595	600	605	1825	
gaa ctt gta gtt gaa gtt aag tca gat aag ctc cca gaa gag atg ggc Glu Leu Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly 610	615	620	1873	
ctc ctg cag ggc agc agc ggt gac aag agg gct ccg gga gac cag gga Leu Leu Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly 625	630	635	1921	
gag aaa tac atc gat ctg agg cat tagccccgaa tcact Glu Lys Tyr Ile Asp Leu Arg His 640	645		1960	
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<211> 646				
<212> PRT				
<213> Homo sapiens				
<400> 4				
Met Gly Leu Pro Arg Leu Val Cys Ala Phe Leu Leu Ala Ala Cys Cys 1	5	10	15	
Cys Cys Pro Arg Val Ala Gly Val Pro Gly Glu Ala Glu Gln Pro Ala 20	25	30		
Pro Glu Leu Val Glu Val Glu Val Gly Ser Thr Ala Leu Leu Lys Cys 35	40	45		

Gly Leu Ser Gln Ser Gln Gly Asn Leu Ser His Val Asp Trp Phe Ser
50 55 60

Val His Lys Glu Lys Arg Thr Leu Ile Phe Arg Val Arg Gln Gly Gln
65 70 75 80

Gly Gln Ser Glu Pro Gly Glu Tyr Glu Gln Arg Leu Ser Leu Gln Asp
85 90 95

Arg Gly Ala Thr Leu Ala Leu Thr Gln Val Thr Pro Gln Asp Glu Arg
100 105 110

Ile Phe Leu Cys Gln Gly Lys Arg Pro Arg Ser Gln Glu Tyr Arg Ile
115 120 125

Gln Leu Arg Val Tyr Lys Ala Pro Glu Glu Pro Asn Ile Gln Val Asn
130 135 140

Pro Leu Gly Ile Pro Val Asn Ser Lys Glu Pro Glu Glu Val Ala Thr
145 150 155 160

Cys Val Gly Arg Asn Gln Gly Tyr Pro Ile Pro Gln Val Ile Trp Tyr Lys
165 170 175

Asn Gly Arg Pro Leu Lys Glu Glu Lys Asn Arg Val His Ile Gln Ser
180 185 190

Ser Gln Thr Val Glu Ser Ser Gly Leu Tyr Thr Leu Gln Ser Ile Leu
195 200 205

Lys Ala Gln Leu Val Lys Glu Asp Lys Asp Ala Gln Phe Tyr Cys Glu
210 215 220

Leu Asn Tyr Arg Leu Pro Ser Gly Asn His Met Lys Glu Ser Arg Glu
225 230 235 240

Val Thr Val Pro Val Phe Tyr Pro Thr Glu Lys Val Trp Leu Glu Val
245 250 255

Glu Pro Val Gly Met Leu Lys Glu Gly Asp Arg Val Glu Ile Arg Cys
260 265 270

Leu Ala Asp Gly Asn Pro Pro His Phe Ser Ile Ser Lys Gln Asn
275 280 285

Pro Ser Thr Arg Glu Ala Glu Glu Glu Thr Thr Asn Asp Asn Gly Val
290 295 300

Leu Val Leu Glu Pro Ala Arg Lys Glu His Ser Gly Arg Tyr Glu Cys
305 310 315 320

Gln Ala Trp Asn Leu Asp Thr Met Ile Ser Leu Leu Ser Glu Pro Gln
325 330 335

Glu Leu Leu Val Asn Tyr Val Ser Asp Val Arg Val Ser Pro Ala Ala
340 345 350

Pro Glu Arg Gln Glu Gly Ser Ser Leu Thr Leu Thr Cys Glu Ala Glu
355 360 365

Ser Ser Gln Asp Leu Glu Phe Gln Trp Leu Arg Glu Glu Thr Asp Gln
370 375 380

Val Leu Glu Arg Gly Pro Val Leu Gln Leu His Asp Leu Lys Arg Glu
385 390 395 400

Ala Gly Gly Gly Tyr Arg Cys Val Ala Ser Val Pro Ser Ile Pro Gly
405 410 415

Leu Asn Arg Thr Gln Leu Val Lys Leu Ala Ile Phe Gly Pro Pro Trp
420 425 430

Met Ala Phe Lys Glu Arg Lys Val Trp Val Lys Glu Asn Met Val Leu
435 440 445

Asn Leu Ser Cys Glu Ala Ser Gly His Pro Arg Pro Thr Ile Ser Trp
450 455 460

Asn Val Asn Gly Thr Ala Ser Glu Gln Asp Gln Asp Pro Gln Arg Val
465 470 475 480

Leu Ser Thr Leu Asn Val Leu Val Thr Pro Glu Leu Leu Glu Thr Gly
485 490 495

Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser Ile Leu
500 505 510

Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser Asn Thr
515 520 525

Thr Thr Gly Leu Ser Thr Ser Thr Ala Ser Pro His Thr Arg Ala Asn
530 535 540

Ser Thr Ser Thr Glu Arg Lys Leu Pro Glu Pro Glu Ser Arg Gly Val
545 550 555 560

Val Ile Val Ala Val Ile Val Cys Ile Leu Val Leu Ala Val Leu Gly
565 570 575

Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys Arg Arg
580 585 590

Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Thr Glu Leu
595 600 605

Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly Leu Leu
610 615 620

Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly Glu Lys
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Tyr Ile Asp Leu Arg His
645

<210> 5

<211> 1962

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:HUMAN MUC18
CDNA, AS MODIFIED TO FACILITATE CLONING

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gtgaaagtgg gcagcacagc cttctgaag tgcggctct cccagtccca aggcaacctc 180

agccatgtcg actggtttc tgtccacaag gagaagcgga cgctcatctt ccgtgtcg 240

cagggccagg gccagagcga acctggggag tacgagcagc ggctcagcct ccaggacaga 300

ggggctactc tggccctgac tcaagtcacc ccccaagacg agcgcacatctt cttgtgc 360

ggcaagcgcc ctcggtccca ggagtaccgc atccagctcc gcgtctacaa agtccggag 420

gagccaaaca tccaggtcaa cccctgggc atccctgtga acagtaagga gcctgaggag 480

gtcgctaccc gtgtagggag gaacgggtac cccattcctc aagtcatctg gtacaagaat 540

ggccggcctc tgaaggagga gaagaacgg gtccacattc agtcgtccca gactgtggag 600

tcgagtggtt tgtacaccc ttgcagtttgc acgtggcac agctggttaa agaagacaaa 660

gatgcccagt ttactgtga gtcactac cggctgccc gtggaaacca catgaaggag 720

tccagggaaatc tccatgtccc tggtttctac ccgacagaaaa aagtgtggct ggaagtggag 780

ccctggaaatc tgctgaagga aggggaccgc gtggaaatca ggtgtttggc tcatggcaac 840

cctccaccac acttcagcat cagcaagcag aaccccagca ccagggaggc agaggaagag 900

acaacccaacg acaacggggc cctggtgctg gagcctgcc ggaaggaaca cagtggcgc 960
tatgaatgtc aggcctggaa cttggacacc atgatatcgc tgctgagtga accacaggaa 1020
ctactggtga actatgtgtc tgacgtccga gtgagtcgg cagccctga gagacaggaa 1080
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<210> 6

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial
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MUC18 FRAGMENT

<400> 6

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28

<210> 7

<211> 27
<212> DNA
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<220>
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MUC18

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27

<210> 8
<211> 5
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<220>
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SEQUENCE ENCODED BY PORTION OF PGEX-6P-1 VECTOR

<400> 8
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1 5

<210> 9
<211> 27
<212> DNA
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<220>
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HUMAN MUC18

<400> 9
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27

<210> 10
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HUMAN MUC18

<400> 10

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25

<210> 11
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<212> DNA
<213> Artificial Sequence

<220>
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SEQUENCE FOR MCU18 CLONED INSERT

<400> 11
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<210> 12
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<212> PRT
<213> Artificial Sequence

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SEQUENCE FOR CLONED MUC18 INSERT

<400> 12
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1 5 10 15

Arg Leu Glu Arg Pro His Arg Asp
20

<210> 13
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:JUNCTION
SEQUENCE FOR CLONED MUC18 INSERT

<400> 13
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<210> 14
<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:JUNCTION
SEQUENCE IN FUSION PROTEIN

<400> 14

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1 5 10 15

Ser Thr Arg Ala Ala Ala Ser
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<210> 15

<211> 78

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:JUNCTION
SEQUENCE IN VECTOR WITH MCU18 CLONED INSERT

<400> 15

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78

<210> 16

<211> 25

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence:JUNCTION OF
FUSION PEPTIDE

<400> 16

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1 5 10 15

Asp Ser Ser Gly Arg Ile Val Thr Asp
20 25